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CHARACTERIZATION AND HABITATS OF BACTERIA AND YEASTS ISOLATED FROM LAKE VANDA IN ANTARCTICA

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Abstract: Several strains of bacteria and yeasts were isolated at various depths of a meromictic lake, Lake Vanda, to clarify their physiological properties in relation to their habitats. Gram-negative rods, strains T-1, T-2, T-6, T-14, 3B, and *Pseudomonas* sp. 3G, were isolated at depths of above 30 m where the temperature is 5–7°C and the chlorinity is low. T-1, T-6, 3B, and 3G grow aerobically below 27°C, optimally about 20°C under low salt concentration (<1% NaCl). Gram-negative aerobic cocci, strains 13A–F, were isolated at a depth of 69 m near the bottom where the temperature is 24°C and the chlorinity is high, up to 76.5 g/kg. Strain 13A grows aerobically at 12–36°C, optimally about 30°C under the addition of 0 to 10% NaCl to the culture medium. T-1, T-6, 3G, and 13A contain more than 81% of unsaturated fatty acids such as oleic acid (18 : 1) and palmitoleic acid (16 : 1). The yeast *Candida* sp. strains T-3, T-4, T-5, T-9, and T-11 isolated at a depth of 5 m grow below 25°C, optimally about 15°C. They are fermentable and can utilize various sugars such as glucose and sucrose. T-5 and T-9 have 74% and 86% of unsaturated fatty acids such as linoleic acid (18 : 2), respectively. The presences of psychrophilic bacteria and yeasts in the water column (5–30 m), and of halotolerant bacteria in the bottom water (69 m) well correspond to the meromictic property of the lake.

1. Introduction

There are a number of ice-free areas, so-called oases in Antarctica, in which numerous lakes and ponds occur having various salt concentrations ranging from snow-melt water to 13 times more concentrated than the seawater (TORII *et al.*, 1988). Although Antarctic environment is extreme for living organisms, the lakes and ponds provide favorable habitats for microorganisms including microalgae. WRIGHT and BURTON (1981) summarized microorganisms in Antarctic fresh-water and saline lakes.

Lake Vanda is a meromictic, saline lake, situated in the McMurdo Dry Valleys of Southern Victoria Land, Antarctica. The lake has been studied by many scientists (*e.g.*, GOLDMAN *et al.*, 1967; TORII *et al.*, 1975; TAKII *et al.*, 1986). The bottom water (69 m)

of this lake has high temperature of about 24°C and high chlorinity (76.5 g/kg), in spite of low temperature (5–7°C) and low chlorinity about 50 m and above in the water column. Dissolved oxygen is saturated or super-saturated above 57 m, but decreases gradually with depth, and anoxic condition appears at about 61 m, where hydrogen sulfide occurs.

Several species of bacteria (MEYER *et al.*, 1962; KRISS *et al.*, 1976), filamentous fungi and yeasts (SUGIYAMA *et al.*, 1967; GOTO *et al.*, 1969) have been found in the lake. These microbial distributions can be expected to be much different with depth, because the physicochemical properties of the lake change largely with depth. MATSUMOTO *et al.* (1984, 1987) suggested the vertical difference of microbial distribution in the lake, based on the concentrations and compositions of organic substances in the water column. Also, TAKII *et al.* (1986) and KONDA *et al.* (1987) show that bacterial numbers increase largely with depth. Few papers, however, have been reported on the characteristics of isolates. We describe here some physiological properties and fatty acid compositions of the strains of bacteria and yeasts isolated at various depths in Lake Vanda, and discuss the relation between microbial properties and the stratification of the lake water.

2. Materials and Methods

2.1. *Samples*

Water samples were collected by G. I. MATSUMOTO on January 2, 1986 from several depths (5 m to 69 m) in the deepest point (69 m) of Lake Vanda (77°32'S, 161°34'E) after drilling into lake ice using a SIPRE ice auger. All the samples were kept at about 4°C during transport by air to our laboratory and until the isolation of microorganisms in April 1986. *Escherichia coli* IAM1016, *Candida albicans* IAM4966, and *Saccharomyces cerevisiae* IAM4512 were provided from the Institute of Applied Microbiology, the University of Tokyo, Tokyo, Japan.

2.2. *Culture and characterization*

Bacterial samples were routinely isolated and cultured on nutrient agar (beef extract, 1%; peptone, 1%; NaCl, 0.5%; agar, 2%) or on 1/100 nutrient agar at 7°C or 22°C aerobically for 12 to 14 days. Isolates were characterized according to COWAN and STEEL (1974), and BUCHANAN and GIBBONS (1974). Yeasts were routinely isolated and cultured on PDA medium (potato, 20%; glucose 2%; agar, 2%), YM medium (peptone, 0.5%; yeast extract, 0.3%; malt extract, 0.3%; glucose, 1%; agar, 2%) or Czapek-Dox-glucose medium ($NaNO_3$, 0.3%; K_2HPO_4, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.5%; KCl, 0.05%; $FeSO_4 \cdot 7H_2O$, 0.001%; glucose, 3%; agar, 2%) at 7°C or 22°C aerobically for 3 to 14 days. Fermentation and utilization of carbohydrates by yeasts were carried out by the methods of IIZUKA and GOTO (1969). Characterization of yeasts was mainly performed according to KREGER-VAN RIJ (1984).

2.3. *Fluorescence microscopy*

Microbial samples were stained with DAPI (4'6-diamidino-2-phenylindole), a fluorochrome specific for DNA, and were observed by a fluorescence microscope (Olympus BHS-RFK) to observe a cell nucleus and/or nucleoids (NAGASHIMA *et al.*, 1984).

2.4. *Fatty acid analysis*

The analytical method for fatty acids was reported elsewhere (MATSUMOTO *et al.*, 1979, 1987). Briefly, strains of bacteria and yeasts collected (*ca.* 0.2–1 g wet weight) were refluxed with 0.5 N potassium hydroxide in methanol (80°C, 2 h). After centrifugation of saponified samples, the supernatants and the residues were acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The extracts were chromatographed on a silica gel column (160×5 mm i.d., 100 mesh, 5% water). Fatty acids were eluted with 3 column volumes of benzene/ethyl acetate (95/5) after elution of hydrocarbons with hexane. The fatty acid fractions were methylated with 14% boron-trifluoride in methanol (80°C, 2 h). Hydrocarbons and fatty acid methyl esters were analyzed using a Shimadzu GCMS-QP1000 gas chromatograph-mass spectrometer. The analytical conditions were as follows: A fused silica capillary column (DB-225, 30 m× 0.32 mm i.d.; film thickness, 0.25 μm) and cooled on-column injection mode were used. Flow rate of helium carrier gas was 4.3 m*l*/min. Column temperature was programmed from 70 to 120°C at 25°C/min, and then 120 to 240°C at 5°C/min. Temperatures of injector, molecular separator, and ion source were maintained at 250°C. Electron impact mass spectra were taken at 70 eV continuously at a scan speed of 1.2 s (m/z 50–550).

3. Results

3.1. *Bacteria*

Gram-negative aerobic rods, strains T-6, T-2, T-1, T-14, 3B, and 3G were isolated at depths of 5 to 30 m in the lake (Table 1). Some bacteria were found in the water samples at depths of 55–65 m, but we could not isolate them. Gam-negative aerobic cocci, strains 13A–F, were isolated at a depth of 69 m. Figure 1 shows phase-contrast

Table 1. Bacteria and yeasts isolated at various depths of Lake Vanda.

Strain	Habitat			Organism*	Shape	Size (μm)	Color**
	Depth (m)	Temp. (°C)	Chlorinity (g/kg)				
T-3	5	5	0.2	Yeast	Oval	6.2×3.9	PRY
T-4				Yeast	Oval	9.6×4.3	PRY
T-5				Yeast	Oval	8.6×5.3	PY
T-9				Yeast	Oval	6.1×4.1	PRY
T-11				Yeast	Oval	6.6×4.0	PY
T-6				Bacterium	Rod	1.5–2.0×0.6	PY
T-1	20	7	0.5	Bacterium	Rod	1.5–4.7×0.5	O
T-2				Bacterium	Rod	1.9–3.4×0.5	O
T-14				Bacterium	Rod	1.4–2.9×0.5	O
3B	30	7	0.5	Bacterium	Rod	1.1–3.4×0.5	O
3G				Bacterium	Rod	1.0–2.5×0.4	YO
13A–F	69	24	76.5	Bacterium	Coccus	0.8–1.2	PY

* Yeast, *Candida* sp.; Bacterium, gram-negative bacterium.
** PRY, pale reddish yellow; PY, pale yellow; O, orange; YO, yellowish orange.

and fluorescence micrographs of the isolates. The rods T-6 are pale yellow, 1.5–2.0 μm long, and 0.6 μm in diameter. Other rods T-1, T-2, T-14, and 3B are orange, similar in cell sizes, 1.1–4.7 μm long, and 0.5 μm in diameter. The rod 3G is 1.0–2.5 μm long and 0.4 μm in diameter. Only 3G has a polar flagellum and shows motility. Each coccus

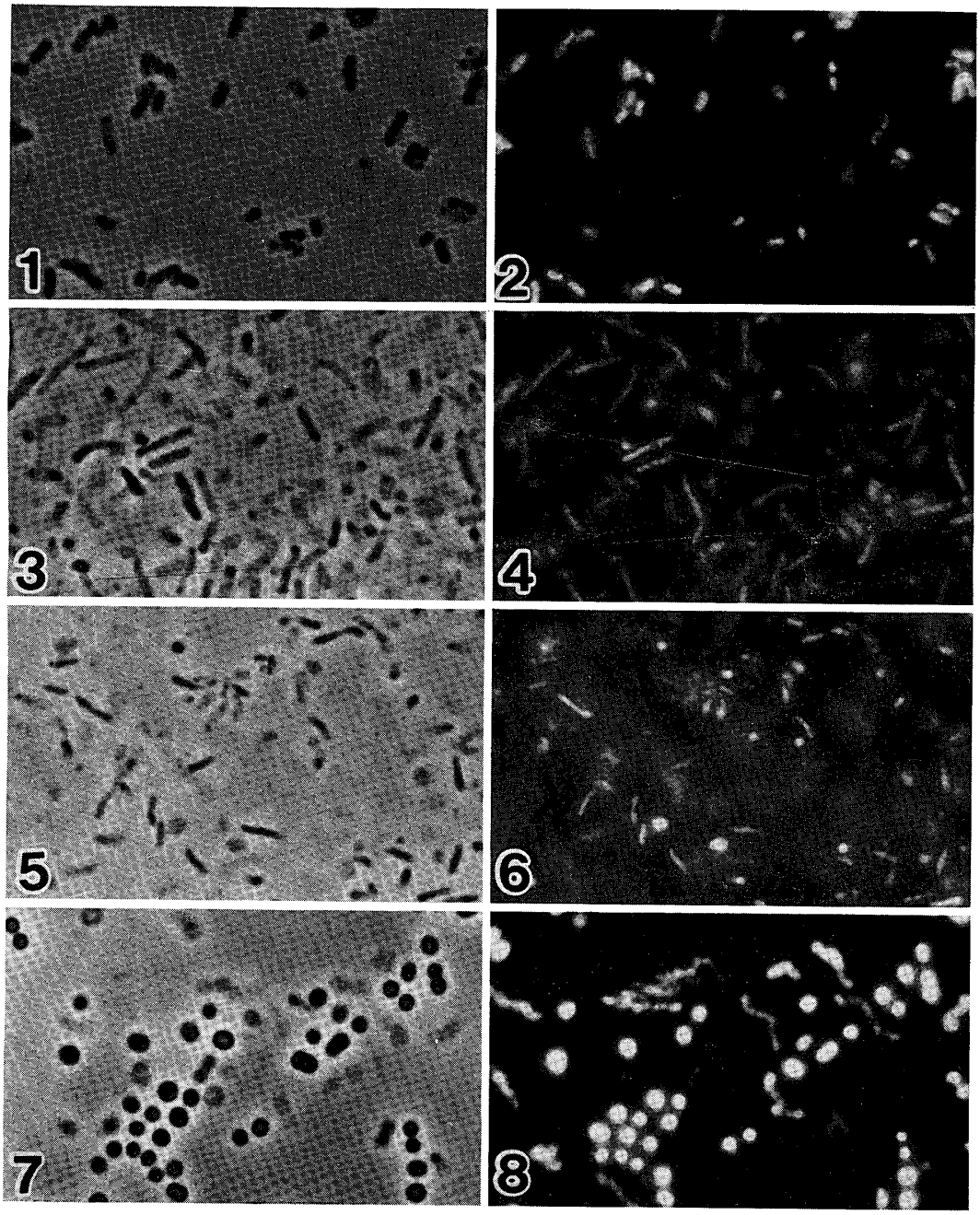

Fig. 1. Phase and fluorescence micrographs of bacteria isolated from Lake Vanda. Phase-contrast micrographs: 1, 3, 5, and 7. Fluorescence micrographs: 2, 4, 6, and 8. 1 and 2, strain T-6; 3 and 4, strain T-1; 5 and 6, strain 3G; 7 and 8, strain 13A. Fluorescence micrographs were taken with ultraviolet (350 nm) irradiation after DAPI staining. A nucleoid DNA can be seen in each vivid bacterial cell. Some string-like features in No. 8 show the traces of the movement of bacterial specimens during the exposure. ×2000.

of 13A–F is about 1.0 μm in diameter. A nucleoid that is a DNA-containing area is recognized in each vivid bacterial cell with a fluorescence microscope (Fig. 1). Strains T-1, T-2, T-6, T-14, and 3B show positive catalase activity and can produce acids from glucose. Strain 3G shows oxydase activity. Strains 13A–F show positive oxydase and catalase activities and can produce a little acid from glucose. According to these tests, the rod 3G was identified as *Pseudomonas* sp. Strains 13A–F are supposed to be the same species.

Figure 2 shows the effect of temperature on the growth of Antarctic bacteria and *E. coli* in culture for 0.25 to 7 days. The rods T-1, T-6, 3B, and 3G (not shown in the figure) are optimum at 22, 20, 18, and 20°C, respectively. The rods cannot grow at higher than 28°C, and they are psychrophilic. The coccus 13A can grow in a wide range of temperature from 12 to 36°C and grows optimum about 30°C. The other cocci 13B–F show similar patterns to that of 13A. They are mesophilic and not as thermotolerant as *E. coli*.

Figure 3 shows the effect of NaCl concentration on the bacterial growth in the culture. The rods T-6, T-1 (not shown in the figure), 3B, and 3G were almost inhibited by the addition of 5% NaCl to the culture medium, while the coccus 13A was tolerant up to 10% NaCl concentration. The strain 13A is more halotolerant than *E. coli*.

Fatty acid compositions of the rods T-1, T-6, and 3G, and the coccus 13A cultured for a week at 20°C, were analyzed by gas chromatograph-mass spectrometry (Table 2). T-1 and T-6 contain almost all oleic acid (18 : 1) and palmitoleic acid (16 : 1), which occupy 98% of total fatty acids. Strain 3G contains 57.7% of oleic acid, 21.7% of palmitoleic acid, 13.9% of palmitic acid (16 : 0). Strain 13A contains 78.2% of oleic acid, 10.1% of palmitoleic acid, and 2.5% of linoleic acid. All strains tested contain more than 81% of unsaturated fatty acids. T-1 is similar to T-6, but is differnt from 3G and 13A in their fatty acid compositions.

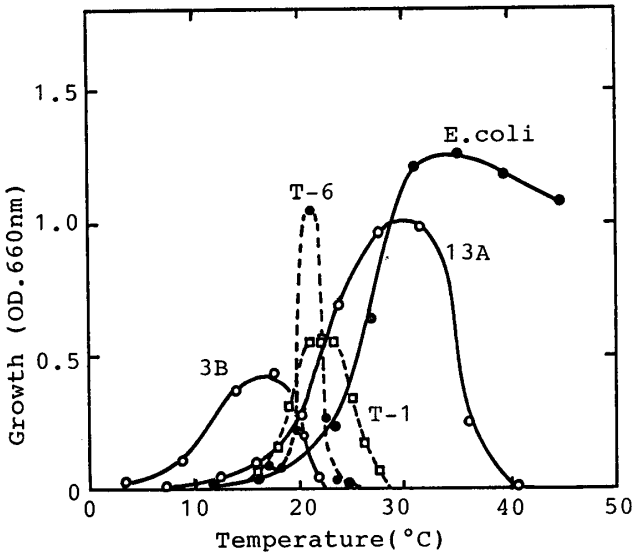

Fig. 2. Effect of temperature on the growth of Antarctic bacteria and E. coli. *Culture period: strains T-1, T-6, and 3B, 5 days; strain 13A, 0.5 day;* E. coli *IAM1016, 0.25 day.*

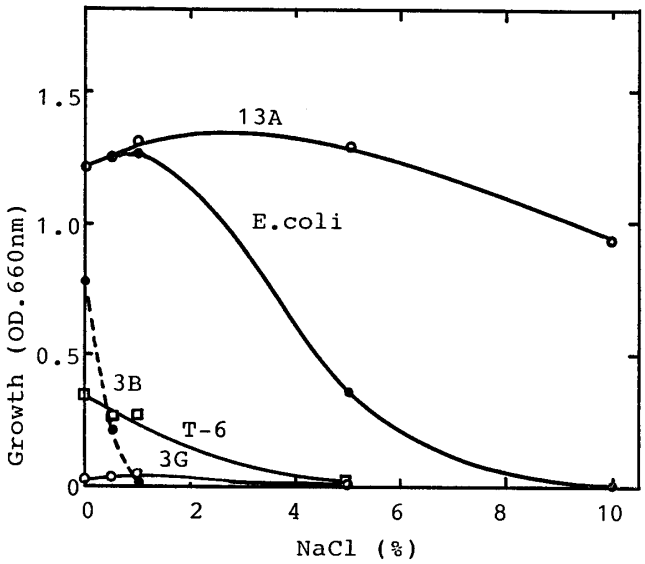

Fig. 3. Effect of the addition of NaCl on the growth of Antarctic bacteria and E. coli. *The strains were cultured at 20°C in the nutrient broth to which various concentrations of NaCl were added. Culture period: strains 3B, 13A, and* E. coli, *5 days; strains T-1, T-6, and 3G, 7 days.*

Table 2. Fatty acid composition (%) of bacteria and yeasts isolated from Lake Vanda.

Fatty acid	Bacterium				Yeast		
	T-1	T-6	3G	13A	T-5	T-9	C.a.*
12 : 0	—	—	—	3.5	—	—	—
14 : 0	—	—	3.7	—	1.4	—	—
15 : 0	—	—	1.4	—	—	—	—
16 : 0	1.7	1.9	13.9	1.9	21.2	13.3	12.3
16 : 1	46.4	38.2	21.7	10.1	2.3	2.0	3.5
17 : 0	—	—	trace	—	0.8	—	1.2
17 : 1**	—	0.9	1.2	2.5	0.6	—	2.5
iso-17	—	—	—	0.6	—	—	—
18 : 0	—	—	—	—	2.3	0.3	0.9
18 : 1	51.9	59.0	57.7	78.2	31.8	17.9	38.7
18 : 2	—	—	0.5	2.5	29.7	40.7	29.3
18 : 3	—	—	—	0.6	9.0	24.5	11.4
Unknown	—	—	—	—	—	0.9	1.3
TUF***	98.3	98.1	81.1	93.9	74.3	86.4	85.4

Bacteria were cultured at 20°C for 5–7 days and yeasts were cultured at 7°C for 5 days.
* *Candida albicans* IAM4966.
** Tentatively identified.
*** Total unsaturated fatty acids.

3.2. Yeasts

Strains of yeasts T-3, T-4, T-5, T-9, and T-11, were isolated at a depth of 5 m in the lake (Table 1). We could not isolate yeasts from any other depths in the lake. Figure 4 shows phase and fluorescence micrographs of vegetative cells of T-5 and T-9.

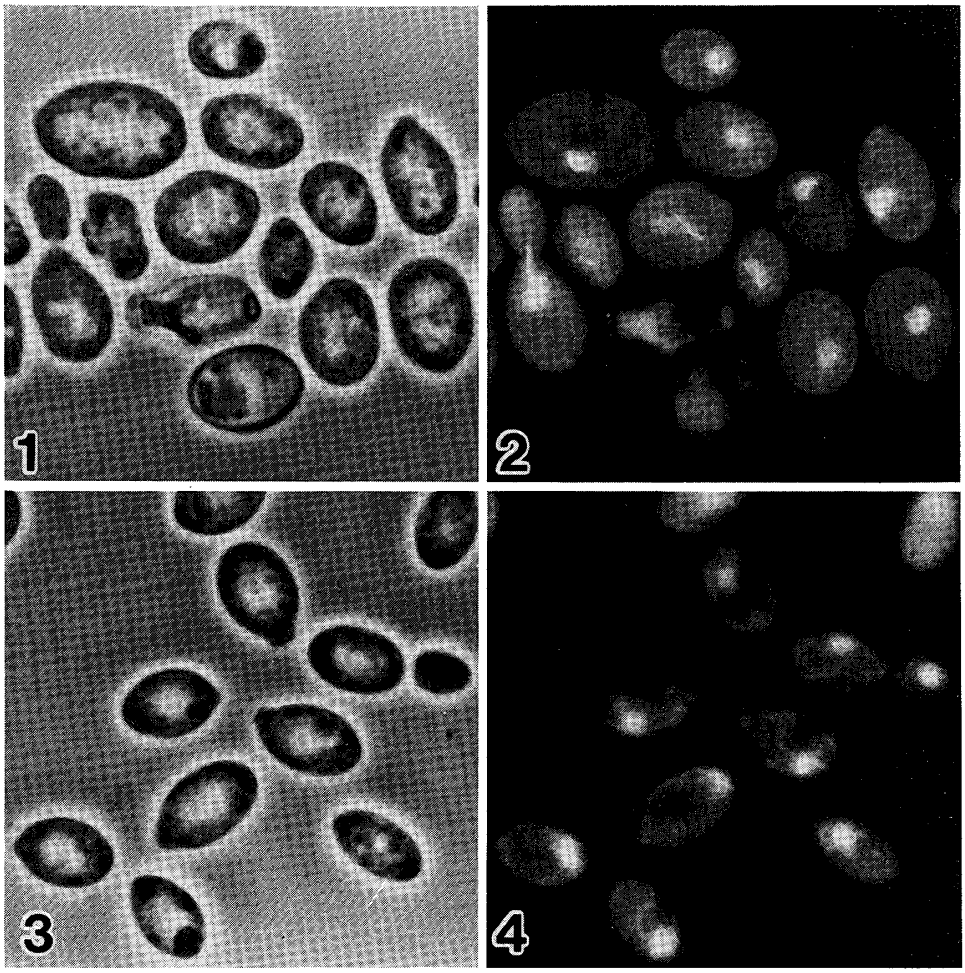

Fig. 4. Phase and fluorescence micrographs of yeasts isolated from Lake Vanda. Phase-contrast micrographs: 1 and 3. Fluorescence micrographs: 2 and 4. 1 and 2, strain T-5; 3 and 4, strain T-9. Fluorescence micrographs were taken in the same manner stated in Fig. 1 legend. A nucleus can be seen in each vivid yeast cell. Mitochondrial nucleoid DNA can be seen sometimes in each cytoplasm. ×2000.

These cells are ovoid or ellipsoidal (globose), 6–9×4–5 μm in diameter. Fluorescence micrographs show the presence of a nucleus and granular or string-like nucleoids of mitochondria in each vivid yeast cell. They have no carotenoids, multiply by multilateral budding, and form pseudomycelium, but not sexual spore. Table 3 shows the fermentation of sugars in Antarctic yeasts, *C. albicans* and *S. cerevisiae*. All strains isolated are fermentative in glucose, galactose, sucrose, maltose, and so on, except for lactose. They are the same as each other, but slightly different from *C. albicans* and *S. cerevisiae*, in the ability of fermentation of sugars. Table 4 shows the utilization of carbohydrates in Antarctic yeasts and *C. albicans*. They can utilize well glucose, galactose, sucrose, maltose, and so on. They are similar to each other, but T-3, T-4, and T-11 are slightly different from T-5 and T-9 in lactose and inositol utilization. There are also some differences between Antarctic yeasts and *C. albicans*. From these data we identified T-3, T-4, T-5, T-9, and T-11 strains as *Candida* sp.

Candida sp. T-3, T-4, T-5, T-9, and T-11 were cultured for 3 days in YM medium

Table 3. Fermentation of sugars in Antarctic yeasts.*

Substrate	Strain						
	T-3	T-4	T-5	T-9	T-11	C.a.**	S.c.***
Glucose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	±	+
Rhamnose	+	+	+	+	+	−	−
Maltose	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	−	−
Melibiose	+	+	+	+	+	−	−
Lactose	−	−	−	−	−	−	−

* Durham's method, 7°C, 24 days. +, positive gas evolution; −, negative gas evolution; ±, a little gas evolution.
** *Candida albicans* IAM4966.
*** *Saccharomyces cerevisiae* IAM4512.

Table 4. Assimilation of sugars and polyols in Antarctic yeasts.*

Sugar	Strain					
	T-3	T-4	T-5	T-9	T-11	C.a.***
Glucose	+**	+	+	+	+	+
Galactose	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	−
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	+	+	−	±	+	±
Melibiose	+	+	+	+	+	−
Raffinose	+	+	+	+	+	±
Arabinose			+	+		−
Mannitol			−	−		+
Inositol	±	±	−	−	−	−
Erythritol	−	−	−	−	−	−

* Condition, at 7°C for 20 days.
** +, positive; −, negative; ±, a little.
*** *Candida albicans* IAM4966.

at various temperature. They can grow from 3 to 25°C and are optimum at about 15°C. Figure 5 shows the curves for T-5 and T-9. In contrast, mesophiles *C. albicans* and *S. cerevisiae* can grow at up to 40°C and they were optimum at about 30°C; therefore, T-3 to T-11 strains isolated are psychrophilic. When NaCl was added at a concentration above 3% to basal YM medium, no strains of yeasts could grow at 7 or 22°C.

Fatty acid compositions of T-5, T-9, and *C. albicans* cultured at 7°C for 5 days were analyzed by the same manner in the case of bacteria (Table 2). They contain mainly oleic acid, linoleic acid, and palmitic acid, which are main components in 23 species of yeasts tested (KANEKO *et al.*, 1976; O'LEARY, 1982). Their unsaturated fatty acid contents amount to more than 74% of total fatty acids. Strain T-9 is considerably different from others in its abundant linolenic acid content (24.5%).

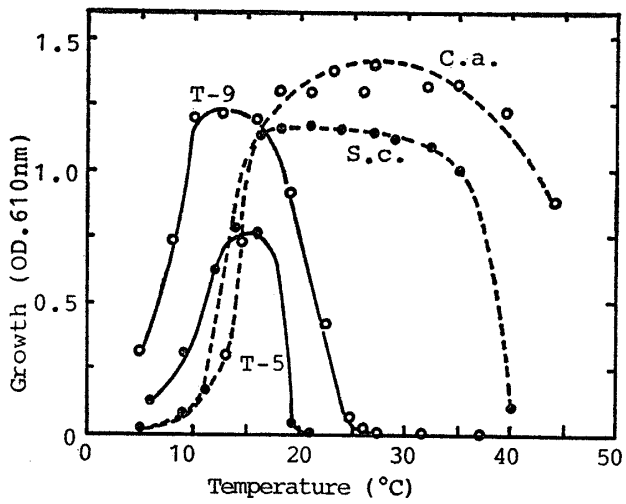

Fig. 5. Effect of temperature on the growth of Antarctic yeasts and C. albicans *and* S. cerevisiae. *The strains were cultured at various temperatures for 3 days in YM medium.*
Abbreviations: C.a., C. albicans; S.c., S. cerevisiae.

4. Discussion

Several species of bacteria, fungi, and yeasts have been found in a meromictic saline lake, Lake Vanda. A few genera of bacteria, *Pseudomonas*, *Chromatobacterium*, *Bacillus*, *Mycobacterium* (KRISS *et al.*, 1976), and *Caurobacter* (TAKII *et al.*, 1986) have been found in the upper layer or ooze of the lake. However, physiological properties of them have been hardly investigated. We could isolate psychrophilic, aerobic rods T-1, T-6, 3B, and *Pseudomonas* 3G from the upper layer of the lake, and halotolerant, aerobic cocci 13A–F strains near the bottom of one layer. The fact that the strains T-1, T-6, 3B, and 3G are psychrophilic, non-halotolerant bacteria well corresponds to their environments at depths of above 30 m (5–7°C, low chlorinity). There is also no discrepancy between mesophilic, halotolerant properties of 13A strain and its environment at a depth of 69 m (24°C; chlorinity, 76.5 g/kg). The distribution of rods in the upper layer and cocci in the lower layer of the lake (Table 1) is well consistent with the observation by TAKII *et al.* (1986). These results indicate that Lake Vanda is a meromictic lake from a viewpoint of the bacterial distribution, as well as the physicochemical environment.

There have been few reports on the isolation of vivid bacteria near the bottom (69 m in depth) of the lake under anaerobic environment. The coccus 13A isolated aerobically from the bottom layer may be a facultative aerobe.

GOTO *et al.* (1969) isolated several yeasts, *Sporobolomyces*, *Cryptococcus*, *Candida*, *Trichosporon*, and *Rhodotorula* from Lake Vanda and reported the presence of a psychrophilic, halotolerant yeast *Candida scottii* that could grow only below 20°C. Here we could isolate psychrophilic, non-halotolerant yeasts, *Candida* sp. strains T-3, T-4, T-5, T-9, and T-11 at a depth of 5 m in the lake (Table 1). The properties of them well correspond to the environment at a depth of 5 m in the lake (5°C, low chlorinity). These yeasts as well as the bacteria isolated have well adapted to the environment of Lake

Vanda. They, therefore, must not be artificial or polluted ones by mankind, but indigenous organisms in Antarctica.

It is well known that some groups of bacteria might be partially characterized by means of their fatty acid composition (LECHEVALIER, 1982). The pattern of fatty acid composition among yeasts is more similar than that among bacteria, but some differences can be seen among several yeasts (KANEKO *et al.*, 1976). As shown in Table 2, fatty acid compositions are different among Antarctic bacteria and yeasts isolated, and *Candida albicans*. Fatty acid analyses may therefore be useful for the identification of microorganisms cultured under the same conditions. We found considerable amount of unsaturated fatty acids such as oleic, linoleic, and palmitoleic acid in these isolates. Strains T-1, T-6, and 13A are especially unique bacteria in Antarctica, because almost all fatty acids (more than 93%) are composed of palmitoleic and oleic acids. The fact may be responsible for the abundance of unsaturated fatty acids in the water column of Lake Vanda (MATSUMOTO *et al.*, 1984, 1987).

Acknowledgments

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